

20.07.00

PCT/EP99/05416  
Micromet GmbH  
Our Ref.: C 2130 PCT

## CLAIMS

1. A multifunctional compound, produceable in a mammalian host cell as a secretable and fully functional heterodimer of two polypeptide chains, wherein one of said polypeptide chains comprises, as the only constant region domain of an immunoglobulin heavy chain the CH1-domain and the other polypeptide chain comprises the constant CL-domain of an immunoglobulin light chain, wherein said polypeptide chains further comprise, fused to said constant region domains at least two (poly)peptides having different receptor or ligand functions, wherein further at least two of said different (poly)peptides lack an intrinsic affinity for one another and wherein said polypeptide chains are linked via said constant domains.
2. The multifunctional compound of claim 1, wherein the functional domains, having receptor or ligand function, are C-and/or N-terminally linked to one or both of said constant immunoglobulin domains.
3. The multifunctional compound of claim 1 or 2, comprising at least three functional domains, having receptor or ligand function.
4. The multifunctional compound of anyone of claims 1 to 3, comprising four functional domains, having receptor or ligand function.
5. The multifunctional compound of anyone of claims 1 to 4, wherein at least two domains, having receptor or ligand function, are N-terminally linked to said constant C<sub>H</sub>1 or C<sub>L</sub> domains.
6. The multifunctional compound of any one of claims 1 to 5, wherein at least one of said domains, having receptor or ligand function, is in the format of a scFv-fragment or a functional part thereof.



7. The multifunctional compound of any one of claims 1 to 6, wherein at least one of said domains, having receptor- or ligand function, is a T-cell co-stimulatory ligand; an antigen binding region specific for a tumor associated antigen, or a proteinaceous compound providing the primary activation signal for T-cells.

8. The multifunctional compound of any one of claims 6 or 7, wherein said scFv fragment or said functional part thereof comprise the  $V_H$  and the  $V_L$  regions of the murine anti-human 17-1A antibody M79, the  $V_H$  and the  $V_L$  regions of the anti-Lewis Y antibody, as shown in Fig. 6, the  $V_H$  and the  $V_L$  regions of the anti-CD3 antibody TR66, and/or the  $V_H$  and the  $V_L$  regions of the human anti-human EpCAM antibody as shown in Figure 55.

9. The multifunctional compound of claim 7, wherein the T-cell co-stimulatory ligand is a cell surface molecule or a fragment thereof expressed on antigen-presenting cells (APC).

10. The multifunctional compound of claim 9, wherein the antigen-presenting cell is a dendritic cell.

11. The multifunctional compound of claim 9, wherein the cell surface molecule is selected from the group consisting of B7-1, B7-2, ICAM-1, ICAM-2, ICAM-3, LFA-3 and CD137-ligand.

12. The multifunctional compound of any one of claims 1 to 5, wherein at least one of said domains, having receptor or ligand function, is an immuno-modulating effector molecule or a fragment thereof.

13. The multifunctional compound of claim 12, wherein said immuno-modulating effector molecule or said fragment thereof is selected from the group consisting of cytokines, chemokines, macrophage migration factor (MIF), T-cell receptors and soluble MHC molecules.



14. The multifunctional compound of claim 13, wherein said cytokine is selected from the group consisting of interleukins, interferons, GM-CSF, G-CSF, M-CSF, TNFs and VEGF.
15. The multifunctional compound of claim 13, wherein said chemokine is selected from the group consisting of IL-8, Eotaxin, GRO $\alpha$ , GRO $\beta$ , GRO $\gamma$ , IP-10, MCP-1, MCP-2, MCP-3, MCP-4, MIG, MIP-1 $\alpha$ , MIP-1 $\beta$ , NAP-2, RANTES, I309, Lymphotactin and SDF-1.
16. The multifunctional compound of anyone of claims 1 to 5, wherein at least one of said domains, having receptor or ligand function, is FAS ligand (CD 95 L) or a fragment thereof.
17. The multifunctional compound of anyone of claims 1 to 5, wherein at least one of said domains, having receptor or ligand function, is a growth factor or a fragment thereof.
18. The multifunctional compound of anyone of claims 1 to 5, wherein at least one of said domains having receptor or ligand function is an angiogenesis inhibitor or a fragment thereof.
19. The multifunctional compound of any one of claims 1 to 18, wherein said constant domain of an immunoglobulin light chain is of the  $\kappa$  type.
20. The multifunctional compound of any one of claims 1 to 19, wherein said constant immunoglobulin domains and said functional receptor-ligand domains are connected by a polypeptide linker.
21. The multifunctional compound of claim 20, wherein said polypeptide linker comprises an Ig-hinge region or a plurality of glycine, alanine and/or serine.
22. The multifunctional compound of claim 21, wherein said Ig-hinge region is an IgG hinge region.

23. The multifunctional compound of claim 22, wherein the IgG hinge region is the upper hinge region of human IgG<sub>3</sub>.
24. The multifunctional compound of any one of claims 1 to 23, wherein said functional domains, having receptor or ligand function, comprise GM-CSF, IL-2 and/or (an) scFv fragment(s) comprising the V<sub>H</sub> and the V<sub>L</sub> regions of the human-anti-human EpCAM antibody, as shown in Figure 55.
25. The multifunctional compound of claim 24, wherein said GM-CSF and said IL-2 are C-terminally linked to said constant C<sub>H</sub>1 or C<sub>L</sub> domains and wherein said scFv fragment(s) comprising the V<sub>H</sub> and the V<sub>L</sub> regions of the human anti-human EpCAM antibody is (are) N-terminally linked to said constant C<sub>H</sub>1 or C<sub>L</sub> domains.
26. The multifunctional compound of any one of claims 1 to 25, wherein said C<sub>H</sub>1 domain is limited to a histidine tag, GST, Staphylococcus protein A, Lex A, a FLAG-tag or a MYC-tag.
27. The multifunctional compound of any one of claims 1 to 26, wherein said functional domains, having receptor or ligand function is or is derived from a non-immunoglobulin domain.
28. A polynucleotide encoding one and/or two polypeptide chains of the multifunctional compound as defined in any one of claims 1 to 27.
29. A vector comprising at least one polynucleotide of claim 28.
30. A mammalian host cell comprising at least one vector of claim 29.
31. The mammalian host cell of claim 30 which is a CHO cell or a myeloma cell.
32. A method of producing the multifunctional compound of any one of claims 1 to 27 comprising culturing the host cell of claim 30 or 31 under conditions that

11 00 00 00 00

allow the synthesis and secretion of said multifunctional compound, and recovering said multifunctional compound from the culture.

33. A composition comprising the multifunctional compound of any one of claims 1 to 27, the polynucleotide of claim 28, or the vector of claim 29 and, optionally, a proteinaceous compound capable of providing the primary activation signal for T-cells.
34. The composition of claim 33 which is a pharmaceutical composition further comprising, optionally, a pharmaceutically acceptable carrier and/or the diluent and/or excipient.
35. The composition of claim 33 which is a diagnostic composition further comprising, optionally, suitable means for detection.
36. Use of the multifunctional compound of any one of claims 1 to 27, the polynucleotide of claim 28 and/or the vector of claim 29 for the preparation of a pharmaceutical composition for preventing and/or treating malignant cell growth.
37. The use of claim 36, wherein the malignant cell growth is related to malignancies of hemapoietic cells or to solid tumors.
38. The use of claim 37, wherein said malignancies of hernatopoietic cells are lymphomas or leukemias.
39. The use of claim 37, wherein said solid tumors are carcinomas, melanomas or sarcomas.
40. A kit comprising the multifunctional compound of any one of claims 1 to 27 and, optionally, a proteinaceous compound capable of providing the primary activation signal for T-cells.

200700

41. The composition of claim 33, the pharmaceutical composition of claim 34, the diagnostic composition of claim 35 or the kit of claim 40, wherein the proteinaceous compound capable of providing the primary activating signal for T-cells is a bispecific antibody interacting with the T-cell antigen CD3.